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MDM2 oncogene as a target for cancer therapy: An antisense approach

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Abstract. The MDM2 oncogene is amplified or overexpressed in human cancers. It has also been suggested that MDM2 levels are associated with poor prognosis of several human cancers. The MDM2 oncoprotein binds to the p53 tumor suppressor protein and serves as a negative regulator of p53. The p53 tumor suppressor also has an important role in cancer therapy, with p53-mediated apoptosis being a major mechanism of action for many clinically used cancer chemotherapeutic agents and radiation therapy. Therefore, the negative regulation of p53 by MDM2 may limit the magnitude of p53 activation by DNA damaging agents, thereby limiting their therapeutic effectiveness. The investigators hypothesize that, by inhibiting MDM2 expression, the MDM2 oncoprotein level will be reduced and the MDM2 negative feedback inhibition of p53 will be diminished, resulting in a significant increase of functional p53 levels that will modulate p53-mediated therapeutic effects. The overall objective of the present study was to investigate the functions of MDM2 oncogene in tumor growth and the potential value of MDM2 as a drug target for cancer therapy. The role of MDM2 in tumor growth is determined by inhibiting MDM2 expression in *in vivo* models of human cancers. The *in vivo* synergistically therapeutic effects of MDM2 inhibition and DNA damaging agents were also evaluated. Significant *in vitro* antitumor activities were found in cell lines, human osteosarcoma SJSA and choriocarcinoma JAR, in a time-, concentration-, and sequence-dependent manner. Following i.p. administration of anti-MDM2 antisense oligonucleotides, *in vivo* antitumor activity was observed in nude mice bearing SJSA and JAR xenografts in a dose-dependent manner. Moreover, *in vivo* synergistically therapeutic effects of MDM2 inhibition and DNA damaging agents adriamycin and 10-hydroxy-camptothecin were observed. This study should provide the basis for future development of anti-MDM2 antisense oligonucleotides as cancer therapeutic agents used alone or in combination with conventional chemotherapeutics.

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Abbreviations: MDM2, mouse double minute 2; Oligo, oligonucleotides; HCPT, 10-hydroxycamptothecin; CPT, camptothecin; FBS, fetal bovine serum; PBS, phosphate-buffered saline; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis

Key words: MDM2, p53, human cancer, apoptosis, chemotherapy

Introduction

The tumor suppressor gene (TP53/P53) product, p53, was first isolated from SV40-transformed rodent cells (1). As a transcription factor, p53 regulates the normal cell cycle by activating the transcription of genes that control progression through the cycle and of other genes that help maintain the genomic integrity of the cells as it coordinates the cellular response to DNA damage by inducing cell cycle arrest or apoptosis (2-6). Alterations of the p53 gene are the most frequent genetic abnormalities in human malignancies (2,3,7-17). Mutations in p53 arise with an average frequency of 50% but the incidence varies from zero in carcinoid lung tumor (13) through 30-86% in breast cancers (14-16) to 97% in primary melanomas (17). Mutations in p53 correlate strongly with a poor prognosis in breast cancer (18), and the level of p53 protein expression may be a predictor of distant metastasis of human breast cancers (19).

Many environmental insults and cancer treatments, including γ-irradiation and chemotherapeutic drugs, increase p53 levels, leading to G1 arrest or apoptosis (20-23). The p53-induced cell growth arrest is due to the ability of p53 to regulate one or more cell cycle checkpoint-related genes. Genes that have been shown to be induced by p53 include MDM2 (24), GADD45 (25), and $p21^{WAF1/CIP1}$ (26). Increased levels of p53, relative to those that induce cell arrest, are necessary to induce apoptosis. Modulating p53-mediated cell arrest and/or apoptosis may lead to the sensitization of tumor cells to DNA damaging agents and radiation therapy. A recent study reveals that p53 gene mutations are common in cancer cell

lines of the National Cancer Institute (NCI) anticancer drug screen: 39 (67%) of 58 cell lines analyzed contain a mutant p53 sequence and the majority of mutant p53 lines express elevated basal levels of the mutant p53 protein (27). These mutant p53 lines tend to exhibit less growth inhibition than wild-type p53 lines following treatment with the majority of 123 clinically used anticancer agents such as bleomycin, 5-fluorouracil, and cisplatin (27). Of the 58 cell lines, 18 (31%) contain wild-type p53 and one (HCT-15) is heterozygous for p53 mutation. This study also demonstrates that cells with wild-type p53 respond better to γ-irradiation and chemotherapy, showing increase in G1 arrest and in expression of p53 reporter genes, MDM2, CIP1/WAF1, and GADD45 (27). These studies suggest that p53 can be a target for improving therapeutic effects of conventional cancer chemotherapy and radiation therapy. P53 has also been suggested as a target for cancer gene therapy (28-32). The transfection of some breast carcinoma, osteosarcoma, colorectal carcinoma and glioblastoma cell lines that have mutant p53 with a wild-type p53 gene significantly suppresses cell growth (28,29). The tumorigenicity of breast cancer cell lines with mutation in both p53 and RB1 (retinoblastoma gene) is reduced by the expression of wild-type forms of either p53 or RB1 (30). The transfection of 9L rat glioblastoma cell line that expresses a mutant p53 with a wild-type p53-expressing plasmid sensitizes the cell to cisplatin treatment *in vitro* and *in vivo* (31). Extensive preclinical studies of p53 gene therapy have been carried out (32).

The MDM2 oncogene was first cloned as an amplified gene on a murine double-minute chromosome in the 3T3DM cell line, a spontaneously transformed derivative of BALB/c 3T3 cells (33). The murine MDM2 protein contains 490 amino acids (human contains 491) and migrates as a 90-97 kDa band on SDS-denaturing gel electrophoresis. It contains a p53 binding domain at the N-terminus, a nuclear localization signal, a central acidic domain and three C-terminal zinc-finger motifs. Overexpression of the MDM2 gene in NIH 3T3 cells confers tumorigenicity (33). The MDM2 gene immortalizes rat embryo fibroblasts and cooperates with the activated *ras* oncogene to transform these cells (34). The MDM2 gene is amplified or overexpressed in about 40-60% of human osteogenic sarcomas and about 30% of soft tissue sarcomas (35,36), indicating that MDM2 oncogene may have a role in tumor development.

The expression of MDM2 is induced by p53 (24,37) and MDM2 binds to p53 with high affinity, inhibiting its ability to act as a transcription factor (38), indicating that MDM2 functions as a negative feed-back regulator of p53. Studies have shown that MDM2 knockout mouse embryos die shortly after implantation; however, mice carrying both inactivated MDM2 and p53 genes are viable (39,40), suggesting that an important function of MDM2 is to negatively regulate p53. In cell culture studies, MDM2 overexpression abrogates the ability of p53 to induce cell cycle arrest and apoptosis (41,42). Studies demonstrate that MDM2 can negatively regulate all known transcription functions of p53 (43). In addition, MDM2 has also been shown to enhance the degradation of p53 by binding to it (44,45), suggesting that it can regulate p53 functions through multiple mechanisms. MDM2 has also been shown to bind to the pRB (46), E2F (47), ribosomal protein L5 (48), and RNA (49) and to regulate the MyoD transcription factor (50). The biological consequences of these activities are not clear but may be associated with transforming properties of MDM2.

Human cancer cell lines or tumor tissues with MDM2 gene amplifications often have wild-type p53 (27,51), presumably inactivated by MDM2, suggesting that inhibition of MDM2 expression in these tumors may lead to activation of p53 and apoptosis of human tumors. It has been demonstrated that many cancer therapeutic agents exert their cytotoxic effects through activation of wild-type p53, and the restoration of wild-type p53 can increase the sensitivity of tumors to DNA-damaging agents (31,32). Restoration of wild-type p53 may also overcome the drug resistance of human cancers associated with disfunction of p53 (52). However, the activation of p53 by DNA damage such as cancer chemotherapy and radiation treatment may be limited in cancers with MDM2, especially those with MDM2 overexpression. Therefore, we hypothesized that inactivation of the MDM2 negative feed-back loop may increase the magnitude of p53 activation following DNA damage, thus enhancing the therapeutic effectiveness of DNA-damaging drugs and radiation therapy. It is also possible to overcome some drug-resistance in tumors with dysfunctional p53. Recently, we have successfully identified an anti-MDM2 antisense phosphorothioate oligodeoxynucleotide that effectively inhibits MDM2 expression in tumor cells containing MDM2 gene amplifications (53,54).

In our laboratories, we have been interested in developing novel genetic-based cancer therapy, with an emphasis on antisense approach (54-56). Antisense oligonucleotides (oligos hereafter) have been shown to be unique research tools in the study of the regulation of gene expression and gene functions. They are also potential therapeutic agents based on rational gene-based drug design. Antisense oligos may achieve their effects by targeting mRNA with which they can hybridize and specifically block protein expression (54,55).

With encouraging results from preclinical and clinical studies of antisense anticancer oligos (57-59) and initial data generated in our *in vitro* studies with anti-MDM2 oligonucleotides (53,54), we are now proposing that specific anti-MDM2 oligos designed with advanced chemistry can be used as a research tool to investigate the role of MDM2 oncogene in the development and treatment of human cancers and, by using *in vivo* approaches, to systematically evaluate these antisense oligos as therapeutic agents used alone or in combination with other therapeutics. These studies will not only provide the proof of principle for anti-MDM2 oligonucleotides but also contribute to the evaluation of the usefulness of antisense therapy in general.

Materials and methods

Test oligonucleotides. The test oligonucleotide, Oligo AS, a 20-mer mixed-backbone oligonucleotide and its mismatch control (Fig. 1) were synthesized, purified, and analyzed using the methods previously reported (57). The purity of the oligonucleotides were shown to be greater than 90% by analyses of ^{31}P NMR, ion exchange HPLC, polyacrylamide gel electrophoresis and melting temperature (both UV and CD), with the remainder being n-1 and n-2 products.

Oligo AS (antisense)	5'-UGACACCTGTTCTCACUCAC-3'
Oligo ASM (mismatch control)	5'-UGAGACCAGTTGTCAGUCAC-3'

Figure 1. Chemical structure of oligonucleotides. N, Phosphorothioate (PS) linkage; N (underlined), 2'O-methyl RNA linkage; N (shadowed). Mismatch control sequences. Oligo AS is a mixed-backbone oligonucleotide, a modification of the previously published PS oligonucleotide (54) with advanced antisense chemistry in order to increase *in vivo* stability, and decreased host toxicity (57).

Chemicals and reagents. Dulbecco's modified Eagle's medium (DMEM) F-12 HAM and phosphate-buffered saline (PBS) were obtained from Sigma Chemical Co. (St. Louis, MO). Fetal bovine serum (FBS), trypsin, penicillin-streptomycin, and trypan blue stain were purchased from Gibco-BRL (Grand Island, NY). Matrigel® basement membrane matrix was obtained from Becton Dickinson Labware (Bedford, MA). Chemotherapeutic agent adriamycin was obtained from Sigma Chemical Co. (St. Louis, MO). Natural product topoisomerase I inhibitor 10-hydroxycamptothecin (HCPT) was obtained from the Midwest Co. (Beijing, China) with the purity of the drug being greater than 98% (60). HCPT has been demonstrated to induce p53, apoptosis and tumor regression (61-63).

Cell culture. The tumor cell lines, SJSA and JAR were obtained from the American Type Culture Collection (ATCC, Rockville, MD) and cultured according the instruction of ATCC. *In vitro* biological activity of oligonucleotides was determined using the conditions described earlier (53,54).

Animal tumor model. Human cancer xenograft models were established using the methods reported previously (61-63). Female nude mice (five-week-old) were purchased from Frederick Cancer Research and Development Center (Frederick, MD) and accommodated for 3 days for environmental adjustment prior to study. Cultured SJSA or JAR cells were harvested from the monolayer cultures, washed twice with DMEM F-12 HAM medium, resuspended in DMEM and injected s.c. ($20x10^6$ cells, total volume 0.2 ml) into the left inguinal area of the mice. The animals were monitored by general clinical observation, body weight, and tumor growth.

In vivo chemotherapy. The animals bearing human cancer xenografts were randomly divided into various treatment groups and the control group (6 mice/group). The oligonucleotides dissolved in physiological saline (0.9% NaCl) were administered by i.p. injection (volume; 5 µl/g body weight). In most cases, the doses were 1, 10, and 25 mg/kg/day, 5 consecutive days per week. The control (non-oligo treated) group received physiological saline only. Tumor growth was monitored using the methods previously reported (61,62). HCPT was suspended in cottonseed oil and given by gavage (volume; 10 µl/g body weight). The doses were 1 and 3 mg/kg/day, 5 consecutive days per week. Adriamycin was given by i.p. injection (volume; 5 µl/g body weight). The doses were 3 mg/kg/day, two doses per week (day 1 and 4 every week).

Western blot analysis. The MDM2, p53 and p21 levels in cultured cells and tumor tissues were analyzed using the methods described previously (53,54,63). In brief, cell lysate or tumor tissue homogenates containing identical amounts of total protein were fractionated by SDS-PAGE and transferred to Bio-Rad Trans-Blot nitrocellulose membranes (Bio-Rad Laboratories, Hercules, CA). The nitrocellulose membrane was then incubated with blocking buffer (PBS containing 0.1% Tween 20 and 5% non-fat milk) for 1 h at room temperature and washed with the washing buffer (PBS containing 0.1% Tween 20) for 5 min twice. The membrane was incubated with primary (anti-MDM2, anti-p53, or anti-p21) antibody overnight at 4°C or for 1 h at room temperature with gentle shaking. The membrane was washed with the washing buffer for 15 min and then twice for 5 min, and then incubated with 1:5000 diluted goat anti-mouse IgG-horseradish peroxidase conjugated antibody (Bio-Rad, Hercules, CA) for 1 h at room temperature. After washing as described above the protein of interest was detected by ECL reagents from Amersham (Arlington Height, IL).

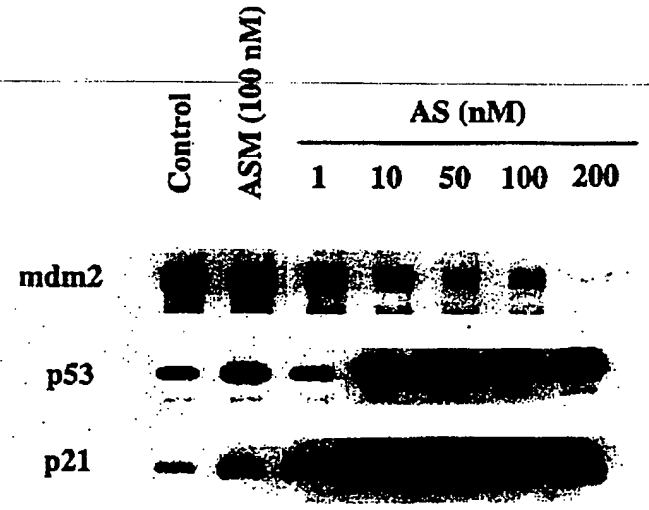

Figure 2. Effects of anti-MDM2 antisense oligonucleotides on MDM2 and p53 protein levels in SJSA cells in culture (A). Cells were incubated with Oligos AS or ASM at various concentrations for 24 h, in the presence of Lipofectin (7 µg/ml). Identical amounts of total protein were analyzed by Western blot using a monoclonal anti-MDM2 antibody. A dose-dependent inhibition of MDM2 expression was observed. At the highest concentration (200 nM), Oligo AS inhibited the MDM2 expression by 95%. In contrast, Oligo ASM had no effect on MDM2 expression.

Results

In vitro inhibition of MDM2 expression by oligonucleotides. As illustrated in Fig. 2, anti-MDM2 oligo, Oligo AS, specifically

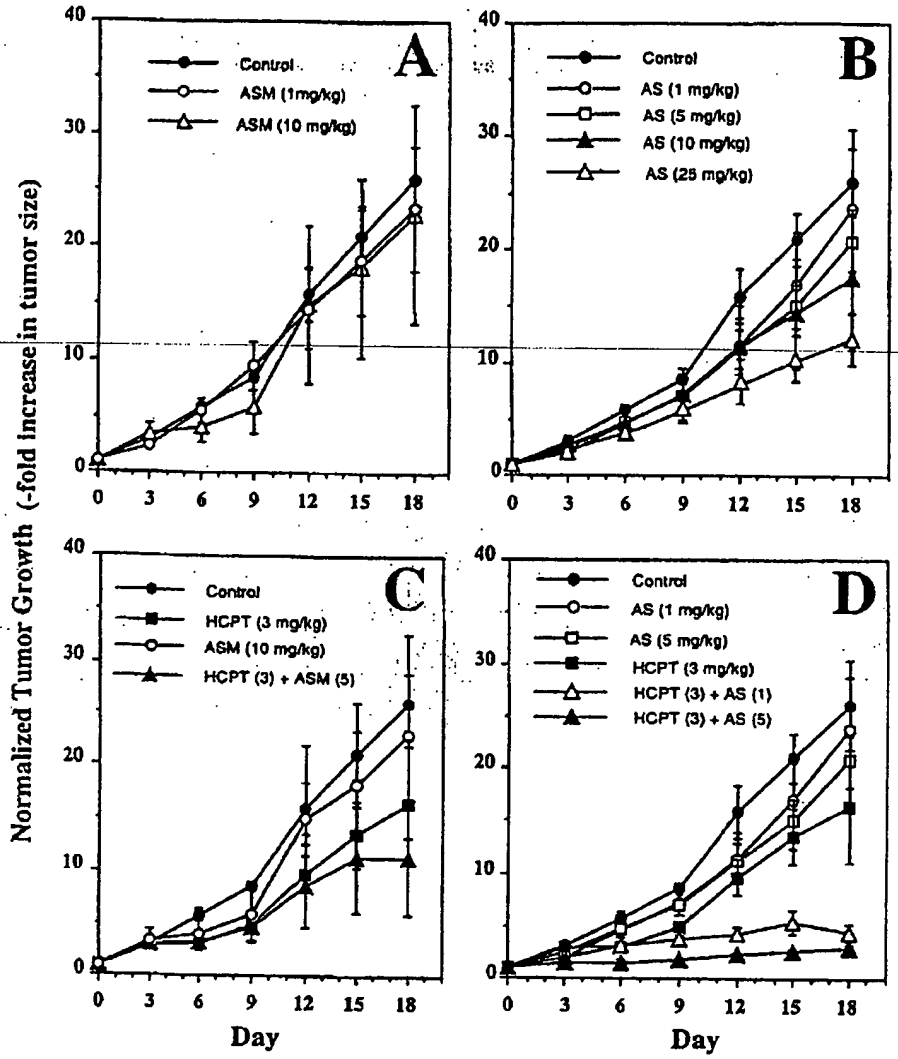

Figure 3. Effects of oligonucleotides on the growth of the human osteosarcoma SJSA xenograft in nude mice. Animals were treated with designated daily doses. Each point represents the mean ± SE of tumor sizes normalized as the fold increases in tumor size (the tumor size at the beginning of the treatment being 1). Panel A, Mismatch control Oligo ASM; Panel B, Anti-MDM2 Oligo AS; Panel C, Mismatch control Oligo ASM in combination with HCPT; Panel D, Anti-MDM2 Oligo AS in combination with HCPT. The animals bearing human tumor xenografts were randomly divided into various treatment groups and the control group (6 mice/group). The oligonucleotides dissolved in physiological saline (0.9% NaCl) were administered by i.p. injection (volume: 5 μl/g body weight). In most cases, the doses were 1, 5, 10, and 25 mg/kg/day, 5 consecutive days per week. The control (non-oligo treated) group received physiological saline only. HCPT was suspended in cottonseed oil and given by gavage at doses of 1 and 3 mg/kg/day, 5 consecutive days per week.

inhibits MDM2 expression in SJSA cells and p53 and p21 levels elevated accordingly. Control oligonucleotide, Oligo ASM had no effect on MDM2, p53 or p21 protein levels. Oligo AS inhibited the growth of tumor cell lines *in vitro* in a dose-dependent manner, with IC_{50} being about 50 nM. The mismatch oligonucleotide, Oligo ASM, had no effect on tumor cell growth.

In vivo chemotherapeutic effects of the oligonucleotide. Fig. 3 illustrates the *in vivo* antitumor effects of the test oligonucleotides on the growth of SJSA tumor xenografts in nude mice. Mismatch oligonucleotide, Oligo ASM, had no significant effect on tumor growth (Fig. 3A). Dose-dependent growth inhibition on SJSA tumor xenografts was found following the treatment of anti-MDM2 antisense oligonucleotide AS (Fig. 3B).

In vivo inhibition of MDM2 expression by oligonucleotides. The anti-MDM2 antisense oligonucleotide, Oligo AS, inhibited the MDM2 expression in SJSA cells *in vivo* in a dose-dependent manner (Fig. 4). The mismatch oligonucleotide, Oligo ASM, showed no effect (Fig. 4).

In vivo synergistic effects between anti-MDM2 oligonucleotides and DNA damaging agents. The test oligonucleotide, Oligo AS, significantly increased the therapeutic effects of HCPT and adriamycin in SJSA xenografts in a dose-dependent manner (Fig. 3D and Fig. 5). The mismatch oligonucleotide,

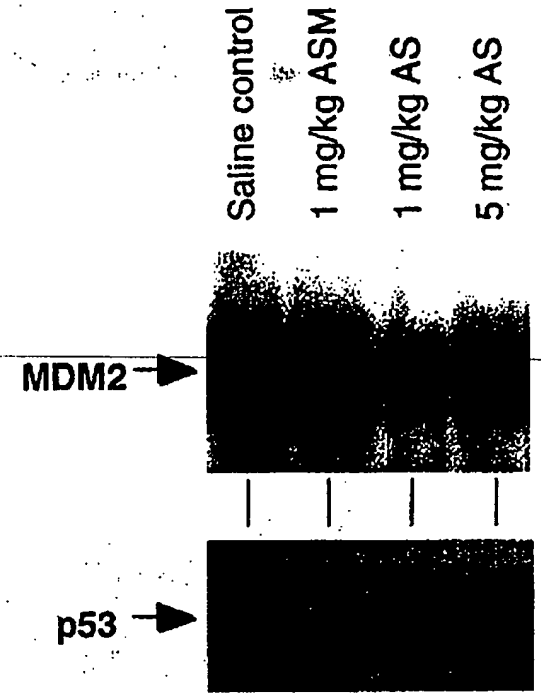

Figure 4. *In vivo* inhibition of MDM2 expression by oligonucleotides. The test oligonucleotide inhibited MDM2 expression in SJSA cells *in vivo*. Animals with SJSA xenografts were treated with Oligo AS or ASM at a daily dose of 1 or 5 mg/kg, 5 days per week for two weeks. The tumors were removed and homogenized. Identical amounts of total protein from the homogenate were analyzed by Western blot using a monoclonal anti-MDM2 or anti-p53 antibody. The mismatch oligonucleotide, Oligo ASM, showed no effect.

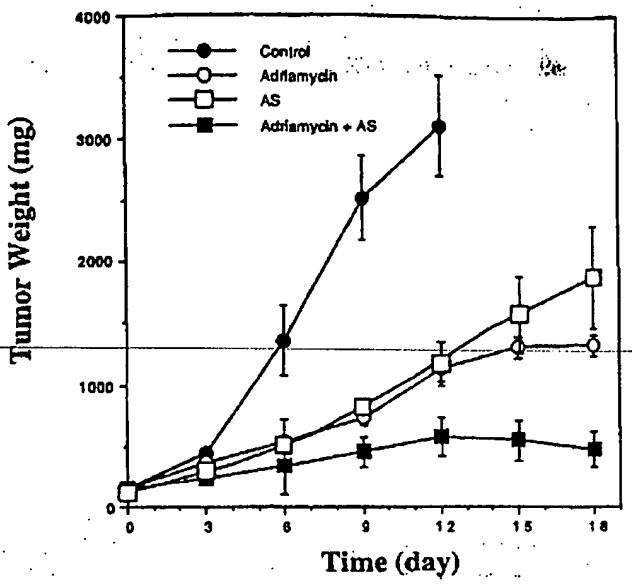

Figure 5. *In vivo* synergistic effects between anti-MDM2 oligonucleotide and adriamycin, a DNA damaging agent. The test oligonucleotide significantly increased the therapeutic effects of adriamycin in SJSA xenograft *in vivo*. The animals bearing SJSA xenografts were randomly divided into various treatment groups and the control group (6 mice/group). The oligonucleotides dissolved in physiological saline (0.9% NaCl) were administered by i.p. injection at the dose of 25 mg/kg/day, 5 consecutive days per week. The control (non-oligo treated) group received physiological saline only. Adriamycin was given by i.p. injection, 3 mg/kg/day, two doses per week (day 1 and 4 every week).

Table I. Therapeutic effectiveness of antisense anti-MDM2 oligonucleotide and HCPT used alone or in combination in nude mice with SJSA xenograft.

Group	Treatment	Tumor growth inhibition (%)	Remarks
1	Control (Saline)	-	
2	Mismatch oligo ASM (1 mg/kg)	9.7	No significant effect
3	Mismatch Oligo ASM (10 mg/kg)	11.8	No significant effect
4	Anti-MDM2 Oligo AS (1 mg/kg)	8.7	No significant effect
5	Anti-MDM2 Oligo AS (5 mg/kg)	21.2	Dose-dependent effect of oligo AS (group 5-7)
6	Anti-MDM2 Oligo AS (10 mg/kg)	32.6	
7	Anti-MDM2 Oligo AS (25 mg/kg)	50.0	
8	HCPT (P.O., 1 mg/kg)	28.0	Significant effect of HCPT
9	HCPT (P.O., 3 mg/kg)	36.2	Significant effect of HCPT
10	HCPT (3 mg/kg) + ASM (5 mg/kg)	56.8	Not synergistic effect
11	HCPT (3 mg/kg) + AS (1 mg/kg)	82.8	Synergistic effect
12	HCPT (3 mg/kg) + AS (5 mg/kg)	88.8	Synergistic effect

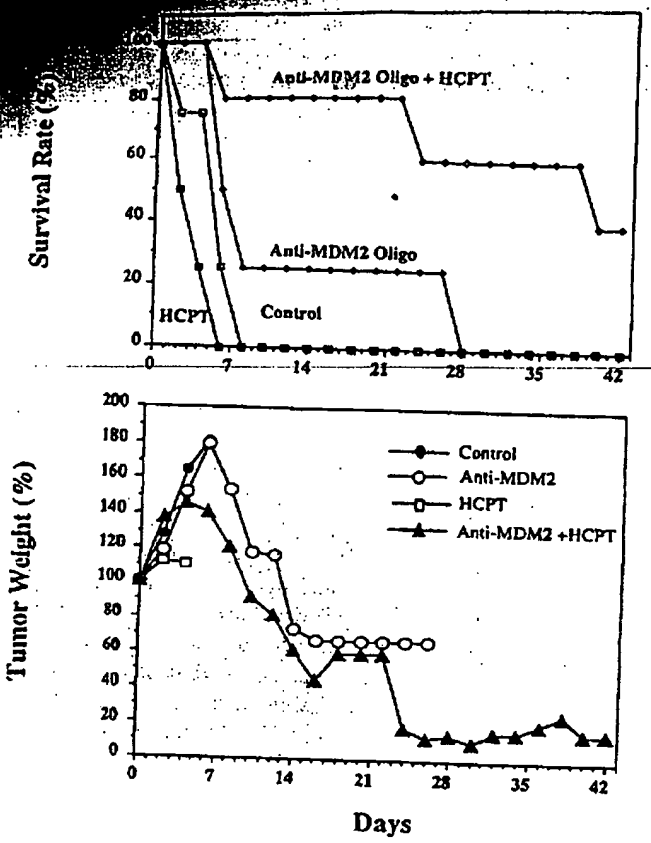

Figure 6. Anti-tumor activities of anti-MDM2 oligo administered alone or in combination with HCPT in JAR model. Animals bearing JAR xenograft (average 2,000 mg) were treated with drugs by direct injection into the tumors at the designated daily dose, 5 doses/week (8 mice/group). Control, Oligo ASM (5 mg/kg/day, 5 injections); Anti-MDM2 Oligo, AS (5 mg/kg/day, 5 injections/week for 3 weeks); Anti-MDM2 oligo + HCPT, Oligo AS 5 mg/kg + HCPT 3 mg/kg (5 injections/week for 3 weeks); HCPT, 3 mg/kg/day (5 injections). Top panel, Survival rates. In control and HCPT groups, all animals died within a week. In the group treated with AS, 25% of animals survived up to 4 weeks. Combinational treatment of anti-MDM2 Oligo AS and HCPT significantly increased the survival rate. Bottom panel, Tumor growth rate. In all animals, tumors grew in the first week of treatment. Regression of tumors were then shown in surviving animals treated with anti-MDM2 oligo alone or in combination with HCPT.

Oligo ASM, showed no effect on the therapeutic effectiveness of HCPT (Fig. 3C). The inhibitory effects on tumor growth of SJSA xenograft of the antisense oligo-nucleotides administered alone or in combination with HCPT are summarized in Table I. These results demonstrate that the combination of MDM2 inhibition and DNA damaging agents has better therapeutic effects than the agents used alone.

In the studies with JAR xenografts, we took a different approach to investigate the effect of anti-MDM2 oligos on tumor regression and animal survival. In this case, we directly injected the Oligo AS and HCPT into large tumors (average 2,000 mg), mimicking the clinical late stage of tumors. The results are depicted in Fig. 6. All control animals died within a week after beginning of treatment. HCPT alone had no effect on animal survival. 25% of animals treated with anti-MDM2 antisense oligonucleotide, Oligo AS, survived up to 4 weeks, accompanied with tumor regression. The combinational treatment of anti-MDM2 oligo AS and HCPT significantly improved the survival rate: 50% of the animals survived over 6 weeks with almost complete tumor regression. These results further demonstrate that MDM2 inhibition directly correlates with tumor regression and animal survival.

Discussion

We and others have suggested that MDM2 oncogene be a target for cancer therapy (53,54,64,65). In previous studies, we developed an antisense anti-MDM2 oligonucleotide that has been shown to specifically inhibit the MDM2 expression *in vitro*. Inhibition of MDM2 expression in cultured human cancer cell lines results in activation of p53 and induces apoptosis or cell arrest (53,54). The antisense oligonucleotides used in our previous *in vitro* studies are PS-oligonucleotides, a class of antisense compounds that have been shown to be associated with various side effects of oligonucleotides *in vivo* (reviewed in refs. 55,56). In the present study, using advanced antisense chemistry (mixed-backbone oligonucleotides), we, for the first time, demonstrated that the anti-MDM2 antisense oligonucleotide, Oligo AS, specifically inhibits MDM2 expression *in vitro* cultured cells and *in vivo* tumor tissues and has significant *in vivo* anti-tumor effects and synergistic effects when used in combination with DNA damaging agents.

The rationale for developing therapeutic strategies targeting at MDM2 oncogene is illustrated in Fig. 7. The p53 tumor suppressor has an important role in inhibition of tumor development and cancer therapy. MDM2 as a negative inhibitor of p53 may have an important role in tumor growth. Inactivation of the p53 feed-back inhibition pathway may lead to growth inhibition or regression of tumor. Our previous *in vitro* data strongly support this hypothesis (53,54). In the present study, we have now used *in vivo* models of human cancers to directly determine the potential therapeutic effects of MDM2 inhibition.

One of the major applications of MDM2 inhibition may be to improve therapeutic effectiveness of cancer chemotherapy such as DNA damaging agents and radiation. The activation or restoration of the wild-type p53 function that leads to apoptosis may increase the efficacy of such treatments, reverse p53-associated drug resistance, or decrease the dose needed, thereby reducing host toxicity. Two chemotherapeutic agents HCPT and adriamycin were be used in the present study to test this hypothesis. Our previous published data show that combining MDM2 inhibition and DNA-damaging treatments (CPT) *in vitro* strongly activate p53 in human tumor cell lines (53). In the present study, we demonstrate that MDM2 inhibition increases the antitumor activity of topoisomerase I inhibitor HCPT in both SJSA and JAR xenograft models in a synergistic manner. These results indicate that inactivating the MDM2 negative feed-back loop with p53 should allow the same level of DNA damage to induce higher levels of p53 activity and apoptosis, thereby increasing therapeutic effectiveness (Fig. 7, panel B).

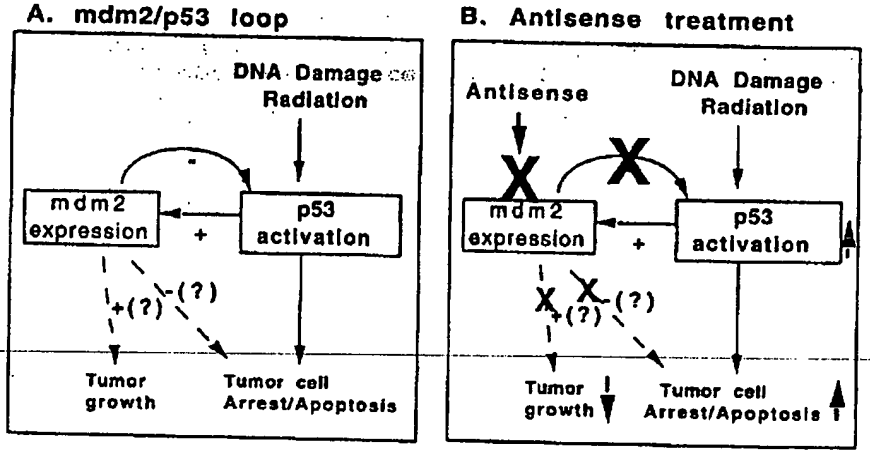

Figure 7. MDM2-p53 interaction as a target for cancer therapy. Panel A, MDM2 as a negative regulator of p53 function. The MDM2 expression is induced by p53 and MDM2 inhibits p53 activity by forming a complex with p53 and may promote p53 degradation. Therefore, in the presence of MDM2, the effect of p53 activation by DNA damaging therapy (e.g., chemotherapy and radiation therapy) is predicted to be limited. In addition, MDM2 overexpression may be associated with promotion of tumor growth through other p53-independent pathways. Panel B, The hypothesized mechanism of action by antisense oligos. Inhibition of MDM2 expression will inactivate the MDM2-p53 feed-back loop, and the inhibitory effect of this pathway can be quantitated by determining the p53 activity. Activated p53 will induce cell arrest and/or apoptosis in tumor cells/tissues. Therefore, it is predicted that tumor growth will be inhibited by anti-MDM2 antisense treatment. It is also predicted that MDM2 inhibition will increase the magnitude of p53 activation by DNA damage and thereby synergistically increase the therapeutic effects of DNA damaging chemotherapeutics. Anti-tumor effects of MDM2 inhibition may be p53-independent *in vivo*.

Alternatively, inhibiting MDM2 expression may allow the same level of p53 activation to be achieved with lower doses of drugs or radiation, thereby leading to reduced host toxicity. MDM2 inhibition may also have application in p53 gene therapy to improve the therapeutic effects.

Acknowledgements

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